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VOLUME XXXIV NUMBER 4

BOTANICAL GAZETTE

OCTOBER, 1902

A MORPHOLOGICAL STUDY OF THUJA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XXXIX.

W. J. G. LAND.

(WITH PLATES VI–VIII)

MATERIAL for the study of *Thuja occidentalis* L. was collected in the vicinity of Chicago during the late spring and early summer of 1901. A few collections were made in June 1902, in order to determine whether the time of fertilization varies in two successive seasons. The trees were visited twice each week and material fixed immediately after the removal of the branches. Branches were also kept in the laboratory, some having water pressure applied to the cut end of the stem, and collections were made daily. All the material from which illustrations are made was taken from a single tree, the most vigorous one to be found. Collections were made from many other trees in order to determine whether the periods of the various stages of development are the same in different individuals of the same locality. Little difference could be observed.

For stages before the formation of archegonium initials 1 per cent. chromo-acetic acid was most useful, but for later stages it was not so good, as it was not possible to secure rapid penetration of the tissues even when used at a temperature of 80°. A modification of Carnoy's formula was found to give the best results for fertilization and succeeding stages, although many fixing agents were given a thorough trial. None of the fixing agents proved entirely satisfactory. Flemming's triple stain gave good results for pollen tubes and early stages in the development of the archegonia, but by far the most satisfactory stain was Haidenhain's iron alum-haematoxylin. Delafield's haematoxylin combined with orange G was best for embryos. Sections were cut 3 to 5 μ thick.

THE POLLEN GRAIN AND POLLEN TUBE.

No attention was given to the formation of microspores from the mother cell, their study being taken up shortly after the separation of the tetrads (*fig. 1*). The appearance of the pollen grains at the time of shedding is shown in *fig. 2*. It does not appear that prothallial cells are cut off. Methods which easily demonstrate the prothallial cells of Pinus and similar forms have failed to show any such structures in Thuja. However, considering the ephemeral nature of prothallial cells in most gymnosperms, and the small size of the microspores of Thuja (20 μ in diameter), it is possible, though not probable, that they may have been overlooked. So far as could be determined, the next step after the separation of the tetrads is the organization of a tube nucleus and a generative cell (*fig. 2*). The microspores of *Cupressus Benthamiana* and of *Biota orientalis* seem in every respect like those of *Thuja occidentalis*.

In material collected June 2, 1901, the pollen tube had penetrated a short distance into the nucellus (*figs. 3–4*). The generative cell had already divided, and both the tube and stalk nuclei were in advance, and close against the large body cell. One of these nuclei is invariably somewhat smaller than the other. In *fig. 4* the smaller nucleus is not shown, as it lies immediately beneath the larger one. Conspicuous protoplasmic streams radiate from the tube and stalk nuclei downward to the end of the pollen tube, but little cytoplasm is found above the body cell. The nucleus of the body cell is in general spherical and is surrounded by a thick sheath of cytoplasm (*figs. 4–5*). A deeply staining spot, presumably kinoplasmic in nature, lies in the cytoplasm. Strasburger (11) shows this spot in his figures of Juniperus, and Chamberlain shows similar structures associated with the male nuclei of *Pinus Laricio.* The finely granular areas in the body cell of Pinus, as described by Miss Ferguson (8), and the kinoplasmic regions described by Coker (5) for the egg of Podocarpus seem to consist of the same substance. The spots, though generally present in the body cell, were not found in the male cells of Thuja. These structures are suggestive of the blepharoplasts of Ginkgo and certain cycads, and may be a survival of some such structures possessed by ancestors from which the Pinaceae have descended.

Before the appearance of the archegonium initials (June 16, 1901), the penetration of the pollen tube is relatively slow, but at this time its downward movement is much accelerated. The tube quickly pierces the remaining rows of nucellar cells, spreads itself over the mouth of the archegonium complex, and absorbs almost entirely the remnants of the neck cells, thus laying bare the upper ends of the eggs (*fig. 10*). The body cell elongates (*fig. 5*) and divides into two hemispherical cells of the same size, this division taking place immediately before fertilization. That both cells are functional is shown by the fact that when only one pollen tube enters the complex two embryos are formed. In many instances it was observed that the contents of the pollen tube, which was in advance of the others, had fertilized two eggs. The equal size of the male cells is another evidence that both are functional.

Arnoldi (1) finds in Cephalotaxus the two male cells of the same size. Strasburger and also Belajeff (3) figure the male cells of Juniperus as equal. Arnoldi's (2) figures of the male cells of Sequoia show them to be equal. According to Coulter (6) the male cells of *Pinus Banksiana* are alike in size and volume. Chamberlain (4) finds the same to be true of *Pinus Laricio.* In cycads male cells are equal. Belajeff (3) observed that in Taxus one of the male cells is much smaller than the other and does not enter the egg at all. Coker (5) finds one functioning male cell in Podocarpus. In *Pinus Strobus* Miss Ferguson (8) reports them as unequal, with the larger cell invariably in advance.

There can hardly be any doubt that the occurrence of two equal male cells is more primitive, and that any difference in size or loss of function is a more specialized condition. It seems reasonable to suppose that both male cells are functional in all forms which have archegonia collected in a complex, for there is nothing to prevent each male cell from reaching an egg. In forms where the male cells are unequal in size the archegonia are scattered, thus making it practically impossible for the second male cell to function, and this will of course account for the reduced size.

Three to four pollen tubes appear to be the normal number in Thuja. They either pursue different routes through the nucellus or pass down side by side. In the latter case, one has its tip in advance of the others. In numerous instances it was observed that when the eggs fail to function, as they frequently do, the pollen tube penetrates to the bottom of the complex and in some instances pierces the jacket cells. When this occurs the male cells increase very much in size as the tube nears the bottom of the archegonium complex (*fig. 7*). In one instance the cells were 50 μ in length. The longer diameter of the hemispherical male cell at the time of fertilization is in general 20 μ.

DEVELOPMENT OF THE ARCHEGONIUM.

Early stages in the development of the endosperm were not studied, but the process seems to be similar to that described by Jäger (9) for Taxus.

The archegonium initials are differentiated about the middle of June, their number being usually six. They rapidly elongate, become conical, and as yet have very little cytoplasm (*fig. 8*). The nuclei move toward the outer end of the initials and the neck cells are cut off (*fig. 9*). So far as could be determined they are cut off simultaneously. Each neck cell divides by anticlinal walls, forming from two to six cells, and there is no periclinal division. Almost immediately after the last anticlinal wall is formed the neck cells begin to disorganize, and they are soon almost entirely assimilated by the central cells, which at this time are increasing in volume at a very rapid rate.

About the time of the cutting off of the neck cells, the jacket cells appear. At first they are poor in contents, but soon become filled with dense cytoplasm, and the adjacent layer of prothallial cells also shows a marked increase in activity. Protoplasmic connections between the jacket cells and the central cells were not seen, but it is possible that they were overlooked owing to the extreme thinness of the dividing wall. It is not believed that the nuclei of the jacket cells pass into the central cells, as reported in Cephalotaxus by Arnoldi (1).

Shortly after fertilization the jacket cells break down, and in many cells the chromosomes become separated, and are then in a favorable condition for counting, the gametophyte number being twelve.

After the neck cell has been cut off, the nucleus of the central cell lies very close to the upper end of the cell (*fig. 10*) and enlarges so much that its longer diameter is sometimes more than four-fifths of the diameter of the egg. A large vacuole (*figs. 10–11*) now rounds out in the center of the cell. Cytoplasmic masses collect at each end of the central cell, and are connected with each other by a thin layer at the periphery of the central cell. Two deeply staining masses, presumably kinoplasmic in nature, are present. The one near the nucleus resembles somewhat the mass mentioned in connection with the body cell, but it is not so sharply limited. The other, in the lower end of the egg, sometimes stains so densely that it often resembles an overstained nucleus.

At this stage the tip of the pollen tube is in the mouth of the archegonium complex, and is separated from the central cells by remnants of the neck cells.

THE VENTRAL NUCLEUS.

Karyokinetic figures showing the cutting off of the ventral nucleus were obtained in material fixed June 20, 1901, and June 16, 1902 (*fig. 12*). It takes a position at one side of and a little above the egg nucleus, almost in contact with the wall of the egg, and usually on the side nearest the center of the archegonium complex. It is not separated from the egg by a wall, but lies free in the cytoplasm of the egg. The figures of Strasburger (**11**) and Belajeff (**3**) show that it takes a similar position in Juniperus.

Arnoldi (**2**) claims that there is no ventral canal cell in the Cupresseae, and among the Taxodieae he failed to find it in Cunninghamia, Sequoia, Cryptomeria, and Taxodium. Coker (**5**), however, finds a ventral nucleus in Taxodium, although it is not separated from the egg by a wall, lying free in the cytoplasm of the egg. He also finds the ventral nucleus very prominent in Podocarpus, but here also no wall is formed. Strasburger (**11**) as long ago as 1879 figured the spindle in Juniperus. There can be no doubt that the free ventral nucleus without a separating wall is a specialized condition. A similar reduction in the neck canal cells can be seen as one traces a series through the bryophytes and pteridophytes. In most of bryophytes the number of neck canal cells is rather large; in the pteridophytes the number has become small, often only two, and even here a wall seldom forms between the two nuclei. In most gymnosperms there is a definite ventral canal cell, cut off by a conspicuous wall; in Taxodium, Podocarpus, and Thuja the nucleus is formed, but the wall is suppressed. This may be the case in the forms described by Arnoldi (**1, 2**), and may have led him to think that the ventral canal cell was not represented. We doubt whether a ventral canal cell is lacking in any gymnosperm, and believe that the absence of such a cell, or at least a nucleus representing it, could be established only by the most indubitable evidence.

When an archegonium has not been fertilized, there are indications that the ventral nucleus divides (*fig. 13*). Coker (5) holds that in Podocarpus these divisions are amitotic. In Thuja after fertilization there are frequent instances of a further division of the ventral nucleus, and these divisions are mitotic. Coulter and Chamberlain (7) suggest that in some cases the ventral nucleus in Pinus may be fertilized instead of the egg. A number of the writer's preparations of Thuja lead him to believe that both the ventral nucleus and the egg in the same archegonium may be fertilized. In *fig. 17* the proembryo is well advanced, while the ventral nucleus has formed a group of four cells — three of which are shown in the figure, as it was drawn from a single section — which certainly suggests a proembryo in the four-celled stage. Another preparation shows eight cells with indications that walls are soon to appear. The probability of such a fertilization is strengthened by finding occasionally in the same ovule embryos growing upward into the nucellus (*fig. 27*), as well as downward into the endosperm. These embryos are so old that it was not possible to determine definitely if their suspensors are connected with a common archegonium, but such appears to be the case. The embryos growing upward into the nucellus are not so vigorous as those growing downward into the endosperm.

FERTILIZATION.

The contents of the pollen tube are discharged into the space immediately above the archegonium complex, but it does not appear that very much enters the egg. Traces of the cytoplasmic contents of the pollen tube can usually be seen in the upper end of the egg, usually showing as densely staining masses. In some cases the tube and stalk nuclei enter the egg, in others only one of these nuclei enters, but in the great majority of cases they do not enter the cytoplasm of the egg at all, but disorganize in the space above the archegonia. *Fig. 14* shows the male cell entering the egg. At this stage, in general, the large vacuole which occupied the center of the egg has disappeared, and the egg nucleus has taken its place. Soon after coming in contact with the egg the male nucleus slips from its sheath and moves downward toward the egg nucleus. The actual breaking out of the male nucleus from its cytoplasmic sheath was not observed, but numerous preparations showed remnants of the sheath in the upper end of the egg. *Fig. 15* shows the male cell just after its escape from the sheath, and also shows one of the rare cases in which the large vacuole is still present at this stage. The male nucleus passes down, and on coming in contact with the egg nucleus presses the side of the latter inward (*fig. 16*), and finally fuses with it. A close series of stages covering the period from the escape of the male nucleus from its sheath to final fusion was not secured. The time from the rupture of the pollen tube to the formation of the fusion nucleus is very short. Ovules taken on June 20, 1901, from a twig bearing perhaps a dozen cones, showed all stages from the formation of ventral nuclei to well developed suspensors. Not infrequently a male cell in contact with the tip of an egg, and proembryos of four cells may be found in the same complex.

THE PROEMBRYO.

The fusion nucleus is in general below the center of the egg, and remains in this position until the first division is completed. The dividing nucleus is surrounded by a mass of deeply staining cytoplasm (*fig. 18*), which as the spindle disappears closes in between the two nuclei and envelops them, as shown in *fig. 19*. The spindle, as in the first division of the egg in other gymnosperms, is intranuclear. When the two nuclei are in the stages shown in *figs. 19–20*, the cytoplasm above begins to thin out, showing in many instances a vacuole-like space between the ventral nucleus and the proembryo. This space is never entirely free from cytoplasmic masses. In some preparations, as well as in all later stages, it appears more like a break in the continuity of the cytoplasm than like a vacuole (*figs. 22–23*).

The two nuclei sink to the bottom of the egg, lose their rounded form (*fig. 20*), and divide simultaneously (*fig 21*); while the ventral nucleus has enlarged considerably (*figs. 22–23*) and is quite conspicuous.

The four nuclei resulting from the second division of the proembryo usually arrange themselves in pairs, as shown in *fig. 22*, which is reconstructed from serial sections. The third division of the proembryo is also simultaneous (*fig. 23*), and gives rise to eight free nuclei, and walls appear shortly after this last division. Among the cycads and in Ginkgo, after fertilization, there is a long period of free nuclear division before any walls are formed. In Podocarpus sixteen or thirty-two free nuclei are formed; and in Taxus and Cephalotaxus a like number. In Thuja the number of free nuclei is constantly eight, while in Pinus and its allies the number is only four. Even so small a number as two is reported by Strasburger (11) as occasionally occurring in Ephedra; and in Gnetum, according to Lotsy (10), there is no free nuclear division at all. It would seem that there is a gradual transition from an extensive free nuclear division to a condition in which the first division of the egg is followed immediately by the formation of a wall. In the development of the embryo Thuja has not advanced so far as forms like Pinus, although in its cyclic arrangement and in the reduction of the male gametophyte it has proceeded much further.

A wall transverse to the long axis of the oospore now separates the eight nuclei into two groups, each group having four nuclei. The nuclei of the lower group are completely walled in, and from these the embryo develops. Walls parallel to the long axis of the oospore extend from the transverse wall upward a short distance into the cytoplasm of the oospore, thus walling in the nuclei on three sides, but leaving them freely exposed to the cytoplasm above (*fig. 24*). The ventral nucleus is still visible but shows marked signs of disintegration.

The four nuclei of the upper group now divide simultaneously, and walls are formed transverse to the long axis of the archegonium (*figs. 24–25*). The proembryo at this time consists of twelve cells; two tiers of four cells each, and the group of four in the tip, which were formed at the time shown in *fig. 23*. The upper tier of incompletely walled nuclei, produced by the division shown in *fig. 24*, soon disintegrate. The second tier rapidly elongates and forms the four suspensors (*fig. 26*).

A single embryo is developed from the lowest group of cells, which is thrust downward by the suspensors. In the Abieteae each cell forms an embryo. Coulter (6) has shown that in *Pinus Laricio* two embryos may develop at the end of a single suspensor. In Thuja there is a tendency to form two groups of cells. The line of division is clearly apparent in the earlier stages of the embryo, especially in cross sections (*fig. 28*). The line of division disappears as the embryo becomes older.

My acknowledgments are due to Professor John M. Coulter, and Dr. Charles J. Chamberlain for assistance rendered during the progress of the work.

THE UNIVERSITY OF CHICAGO.

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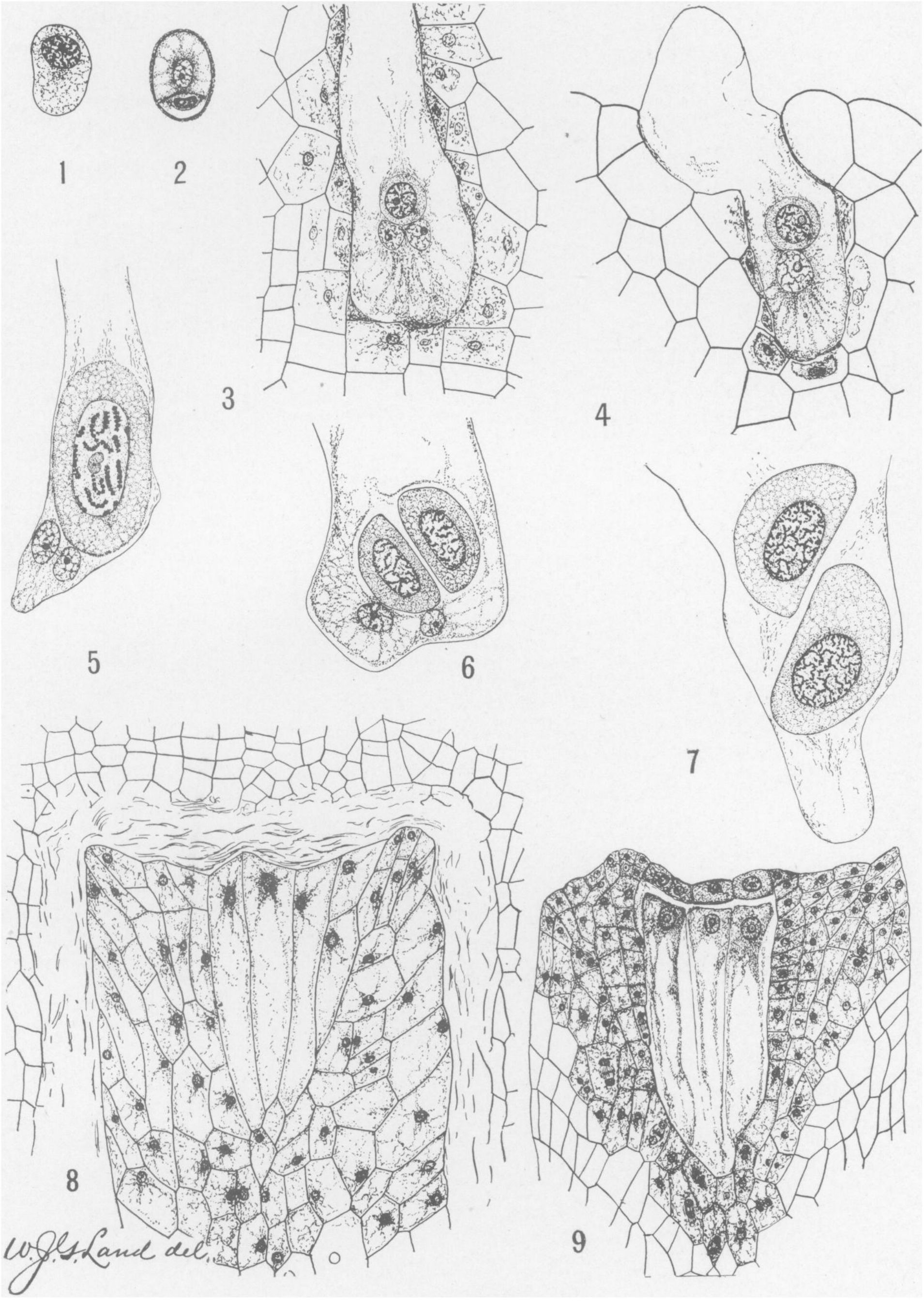
EXPLANATION OF PLATES VI–VIII.

The figures, with the exception of *figs. 8, 9, 10, 11*, and *28*, were drawn with Abbé camera lucida, Bausch and Lomb $\frac{1}{12}$ objective and ocular 2. For *figs. 8, 9, 10, 11*, and *28* a Bausch and Lomb $\frac{1}{6}$ objective and ocular 2 were used. The magnification of *figs. 8, 9, 10, 11*, and *28* was 450; all others 850. The plates are reduced to one-half the original size. The abbreviations used are: *b*, body cell; *v*, ventral nucleus; *m*, male cell; *o*, egg nucleus; *s*, suspensor



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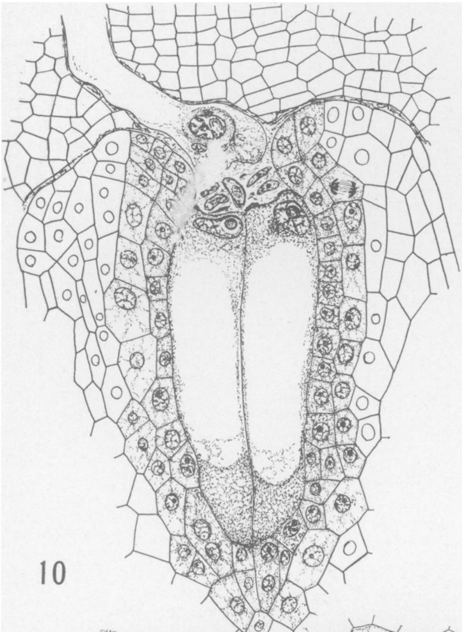

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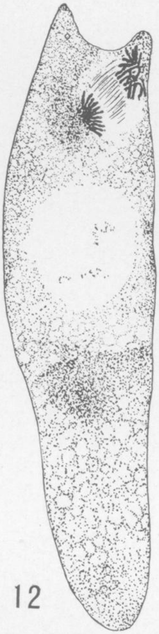

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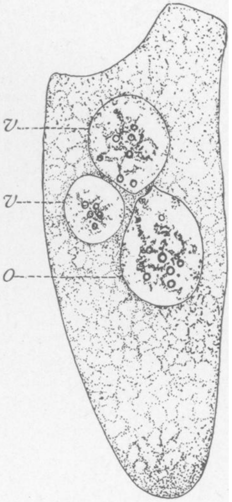

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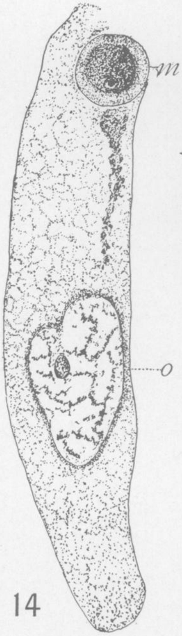

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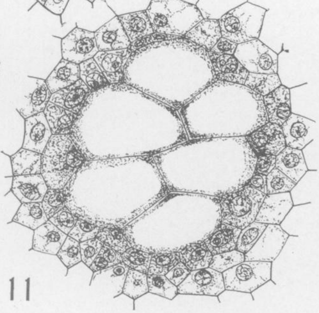

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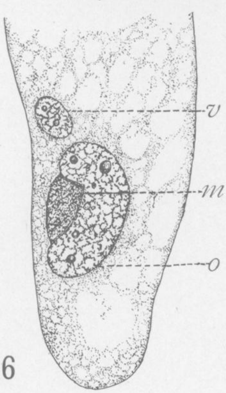

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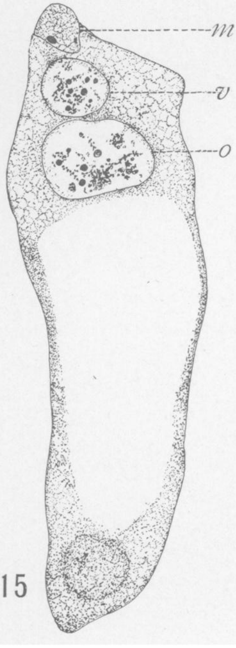

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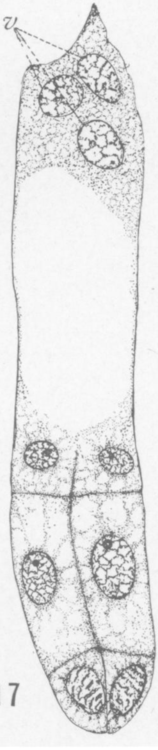

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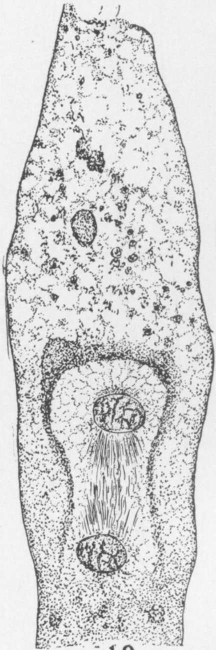

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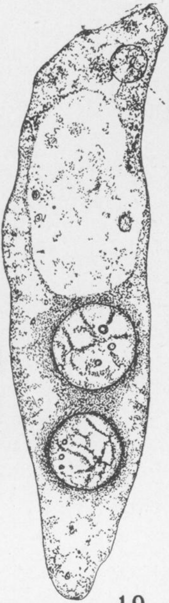

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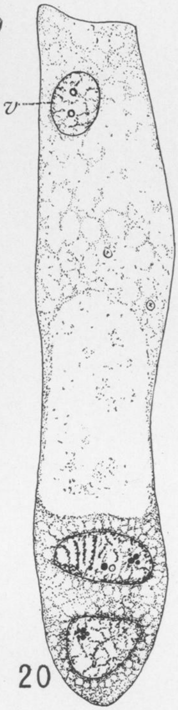

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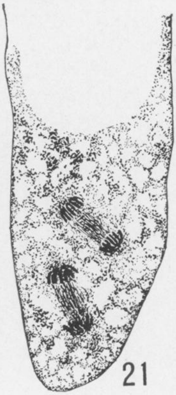

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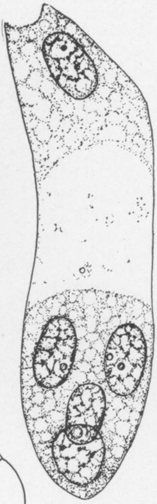

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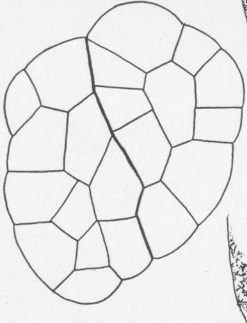

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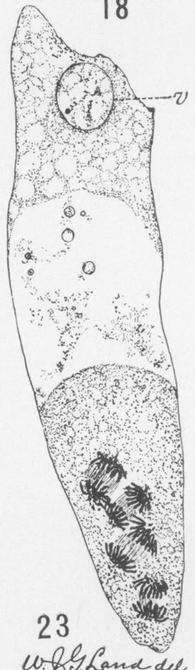

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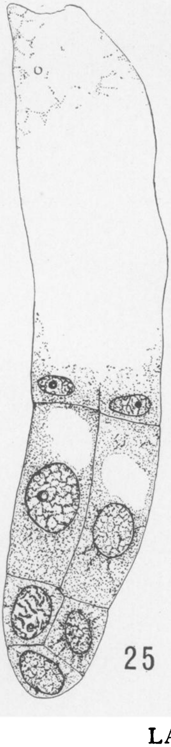

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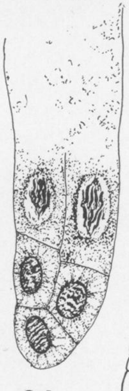

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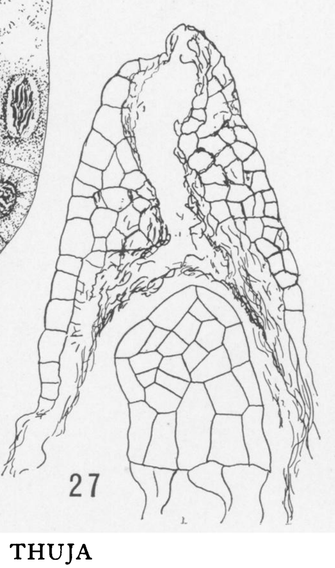

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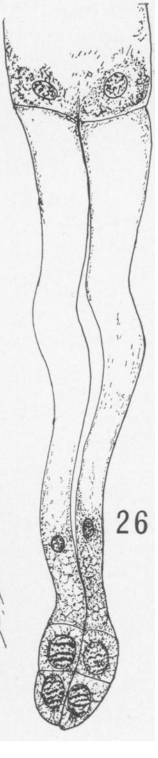

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FIG. 1. Pollen grain shortly after separation of tetrad.

FIG. 2. Pollen grain showing tube nucleus and generative cell.

FIG. 3. Pollen tube with tube and stalk nuclei in advance of the body cell.

FIG. 4. Pollen tube entering nucellus.

FIG. 5. Body cell preparing to divide.

FIG. 6. Tip of pollen tube with two male cells and tube and stalk nuclei.

FIG. 7. Male cells at the bottom of an archegonium which failed to function.

FIG. 8. Longitudinal section through upper end of prothallium, showing archegonium initials.

FIG. 9. Archegonium complex after neck cells have been cut off.

FIG. 10. Archegonium complex just before cutting off the ventral nucleus; jacket cells very active, neck cells disintegrating, and body cell preparing to divide.

FIG. 11. Transverse section through middle of an archegonium complex, showing the large vacuoles.

FIG. 12. Cutting off the ventral nucleus.

FIG. 13. Probable division of the ventral nucleus.

FIG. 14. Male cell entering the egg.

FIG. 15. Male nucleus after slipping out of its cytoplasmic sheath.

FIG. 16. Male and female nuclei in contact.

FIG. 17. Division of ventral nucleus in upper end of oospore; only three of the four nuclei shown; below is the proembryo, with the suspensors beginning to elongate

FIG. 18. Division of the fusion nucleus.

FIG. 19. The two nuclei resulting from the division of the fusion nucleus; the ventral nucleus is above.

FIG. 20. The two nuclei at the bottom of the egg.

FIG. 21. Second division of the proembryo.

FIG. 22. Four nuclei resulting from the second division; the ventral nucleus above.

FIG. 23. Third division of the proembryo; this figure shows the last simultaneous division of the nuclei.

FIG. 24. Upper tier of nuclei dividing to form suspensors.

FIG. 25. Second tier elongating into suspensors.

FIG. 26. Suspensors, with embryo.

FIG. 27. The most advanced one of a number of embryos growing upward through the nucellus; in the preparation from which the figure is drawn there are the usual number of embryos growing downward.

FIG. 28. Transverse section of an embryo, showing bipartite appearance.